3612-Pos Board B473

Imaging Molecular Transport Across Lipid Bilayers

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The passive transport of small molecules across the plasma membrane is a key physiological process. Literature measurements of membrane permeability to small molecules have varied widely. We used confocal microscopy to image the transport of molecules into a giant unilamellar lipid vesicle (GUV). Fluorescent dyes were used to trace the transport of molecules. The GUV was immobilized on the surface of a microfluidic channel by biotin-avidin binding. This microchannel allows the rapid and uniform exchange of the solution surrounding the GUV. Using a spinning-disk confocal microscope, the entire concentration field is captured in a short exposure.

We used this system to study the passive transport of carboxylic acids, which have many properties common to small-molecule drugs. The transport of these acids across cell membranes has been widely studied, but there is much variation in the reported permeabilities. By using pH-sensitive fluorescein-dextran to track the acids permeating through the GUV membrane, our results showed that more lipophilic acids cross the bilayer more quickly. A finite difference model was developed to simulate the experimental process and derive precise permeability values. The permeabilities change with the same trend as oil-water partition coefficients, demonstrating that Overton's rule applies to this class of molecules.

We used the imaging technique described above to study the transport of protons across compositionally asymmetric lipid bilayers. Synthetic asymmetric GUVs were prepared via a microfluidic multiphase droplet flow technology to mimic membrane charge asymmetry. Negatively charged phosphatidylserine was added to an asolectin GUV on either the internal or external leaflet. The permeation rates of protons into and out of these GUVs were measured. The proton distribution across the asymmetric GUV membrane at equilibrium was also studied. This research can reveal how asymmetric cell membrane composition affects small molecule transport behavior in physiological processes.

3613-Pos Board B474

Molecular Mechanisms of Proton Permeation across Lipid Membranes-Effect of Cholesterol

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Despite years of study the mechanisms by which H^+ permeate lipid membranes are not well understood [1, 2]. H^+ flux differs from that of other ions in that H^+ conductance is not dependent on the actual $[H^+]$ in the solution. Combining careful permeability measurements with structural analyses of lipid bilayers using X-ray diffraction, we have developed models of water, solute and H^+ permeation across membranes; the models include various headgroups, chain lengths and extent of unsaturation[3,4]. We compared H^+ permeability with physical parameters of the lipids, such as area/lipid, hydrocarbon thickness, bending modulus and compressibility modulus. Similar to water and solutes, in membranes composed of a single phospholipid, H^+ permeability varied linearly with area/lipid, and was unrelated to other physical parameters. On this basis, in single component lipid systems, the rate limiting step for H^+ permeation is hypothesized to be penetration of the proton from the aqueous medium into the lipid bilayer. When cholesterol is a component of the bilayer, water permeability decreases ($15.8 \pm 0.58 \times 10^{-3}$ cm/s in absence and $6.8 \pm 0.57 \times 10^{-3}$ cm/s in presence of cholesterol respectively). In contrast H^+ permeability increases as the proportion of cholesterol increases in the bilayer (0.056 ± 0.006 cm/s in absence and in presence of cholesterol 0.113 ± 0.005 cm/s). We conclude that mechanisms of H^+ permeability differ markedly from those of water and solutes. We have developed and are testing a new model for H^+ permeation, which defines how cholesterol enhances H^+ flux, while impeding water and solute fluxes.

3614-Pos Board B475

Analysis of Aquaporin Facilitated CO_2 Diffusion Across Biological and Artificial Membranes

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The contribution of membrane proteins to membrane diffusion of CO_2 in biological systems has been questioned for two major reasons: the violation against the Meyer-Overton rule and the disregarding of unstirred layer effects. According to Meyer-Overtons rule membrane diffusion of gasses is not limited by the membrane itself nor can it be improved by membrane proteins. Due to theoretical considerations and experimental evidence it was concluded that the solubility diffusion model has to be applied for CO_2 membrane diffusion.

We studied membrane CO_2 flux ($J_{M,CO2}$) into *Arabidopsis* mesophyll cells using a scanning pH microelectrode, which has been shown to be sensitive enough to detect possible limitations by unstirred layers. *Arabidopsis thaliana* mesophyll cells were exposed to saturating light intensities to trigger photosynthesis and induce cellular CO_2 uptake. The data indicate that under these conditions J_{CO2} of mesophyll cells depends on the expression of the aquaporin AtPIP1;2. Inhibition of a different aquaporin (AtPIP2;3) did not modify $J_{M,CO2}$. It can be concluded that unstirred layers are not rate limiting for cellular CO_2 uptake of *Arabidopsisthaliana* mesophyll cells, but the expression of the integral membrane protein AtPIP1;2. The results provide new arguments to the ongoing debate about the validity of the lipid bilayer model system and the Meyer-Overton rule for cellular gas transport and suggest a different view on molecular gas transport mechanisms of living cells.

3615-Pos Board B476

Probing Gas Diffusion Pathways in Cytochrome C Oxidase with Explicit and Implicit Ligand Samplings

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Cytochrome C oxidase (CCO) couples reduction of O_2 to water with proton pumping across the membrane, thereby generating a driving force for ATP synthesis. The x-ray structures of A-, B- and C-type CCOs suggest that an elongated hydrophobic cavity connecting the membrane core to the protein's active site might serve as an O_2 access point to the catalytic active site of the enzyme. The structure of this cavity is, however, varied in different CCOs. While B- and C-type CCOs exhibit two entrances into this cavity, A-type CCOs appear to only have one entrance. To investigate the involvement of the hydrophobic cavity in O_2 diffusion and to identify (potential) additional O_2 entry pathways, we employed two complementary approaches using molecular dynamics simulations, performed on membrane-bound models of various CCO isoforms. In one approach, using a large ensemble of equilibrated protein conformations collected in the absence of O_2, free-energy of O_2 insertion over a grid covering the entire protein matrix is calculated using the ''implicit ligand sampling'' method. In the other approach, we included O_2 molecules explicitly in the simulations and monitored their diffusion through the system. We observed favorable O_2 binding and rapid O_2 diffusion primarily from the membrane core, characterizing the hydrophobic cavity as a major O_2 delivery pathway. Moreover, through simulations performed on a mutant enzyme, we identify a site that may contribute to the experimentally observed diffusion-controlled O_2 binding kinetics in B-type CCO from *Thermus thermophilus*.

3616-Pos Board B477

Unassisted Transport of Block Tryptophan through DOPC Membrane: Experiment and Simulation

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The permeation of a blocked tryptophan through a DOPC bilayer membrane is investigated to probe unassisted or passive transport. The transport rate is measured experimentally and modeled computationally with atomically detailed simulations using the Milestoning algorithm. The time scale measured by PAMPA experiments is ~8h. Simulations with Milestoning suggest a Mean First Passage Time (MFPT) of ~4h. A similar calculation with the solubility-diffusion model yields MFPT of ~15min. Both methods show significant variations for the computed permeation rate from opposing sides of the membrane (a factor of about 100). This underlines the difficulties in sampling membrane conformations even after 50 nanoseconds of umbrella sampling simulations. The permeation rate for this small peptide is very different (nine orders of magnitude slower) from the permeation rate of a tryptophan side chain only that was computed previously. This difference suggests critical dependence of transport time on permeant size and on hydrogen bonding. Analysis of the simulation